

Mix. (C. L.)

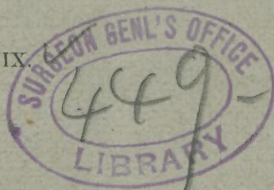
CONTRIBUTIONS

FROM THE

CRYPTOGAMIC LABORATORY OF HARVARD
UNIVERSITY.

XVI. ON A KEPHIR-LIKE YEAST FOUND IN THE
UNITED STATES.

By CHARLES L. MIX.



[REPRINTED FROM THE PROCEEDINGS OF THE AMERICAN ACADEMY
OF ARTS AND SCIENCES, VOL. XXVI.]

presented by the author

IX.

ON A KEPHIR-LIKE YEAST FOUND IN THE
UNITED STATES.

BY CHARLES L. MIX. ✓

Presented by Professor W. G. Farlow, May 26, 1891.

A SPECIES of yeast which causes alcoholic fermentation of milk is well known in Europe, the attention of the leading scientists having been called to it by Edouard Kern, in an article published during November, 1881, and entitled, "Ueber ein neues Milch-ferment aus dem Kaukasus." In order to give an intelligible description of a similar ferment which exists in the United States, a summary of Kern's paper becomes a necessity.

Kern's milk-ferment is found in the region of the Caucasus Mountains, and so far as is known in no other place. It is called by the Caucasian peasants "kephir," "kiphir," "kiaphir," or "kefir." The country being a mountainous one, agriculture is impossible, so that milk and flesh are the food of the peasants. However, they do not drink their milk fresh, but ferment it, adding to it what are known as "kephir-grains" in the proportion of one volume of the grains to six or seven volumes of milk. The whole is then exposed to the air for twenty-four hours at an ordinary temperature, and shaken frequently. The "ferment-milk" thus formed is poured from the grains and mixed with twice its volume of fresh milk, which it ferments in turn, eliminating a large amount of carbonic acid gas, and forming from $\frac{1}{2}\%$ to 1% of alcohol. When kephir is made successfully, it is a thick fluid without any very large coagulated clumps, and with a pleasantly acid taste; by longer fermentation it becomes a frothing, foaming, strongly acid drink, like the koumiss of the Steppes.

According to Kern, this ferment is used not only as a drink, but also as a curative for various diseases, with great success, various gastric and pulmonary complaints, it is said, being cured by it. Its reputation, Kern continues, has extended beyond the narrow limits of the mountainous region where it originated, and has already reached many cities of the Caucasian district.



Examined with the eye, the kephir-grains when fresh are found to consist of white, compact, elastic masses, enveloped by a slime, and with a spherical or elliptical contour, varying from 1 mm. to 5 cm. in diameter. The very small grains have a smooth spherical exterior, while the larger ones are provided with outgrowths and furrows, looking more or less like a very small cauliflower. When the grains are dried they assume a yellowish brown color, and shrink a good deal by the loss of water. When examined with the microscope there are found in each grain, whatever its form or size, two different structures, yeast cells and Bacteria. The latter form the mass of the grain in which the yeast cells are embedded.

The yeast cells occur in pairs or rows of cells of all shapes and sizes. Most of them are elliptical or spherical, the former varying from $3.2\ \mu$ to $9.6\ \mu$ by $3.2\ \mu$ to $6.4\ \mu$, the spherical ones varying from $3.2\ \mu$ to $6.4\ \mu$ in diameter. Each yeast cell has a plainly visible membrane with a double contour, brought out by stains. Within the cell is a vacuole, at the poles of which are often found small fat globules in no definite number, but which increase in number as the cell is dried, the protoplasm at the same time becoming granular, the vacuoles diminishing in size and ultimately disappearing. The yeast cells increase by budding.

Kern discusses the question of the possibility that the yeast cells may be the spores of some *Mucor*, as *M. racemosus* for example, since these are known to cause alcoholic fermentation; but since cultures continued for weeks failed to show him a trace of mycelium, he concludes that there can be no doubt that these are true yeast cells.

The origin of the kephir grains was unknown to Kern. He could find no wild form of yeast from which they might have been cultivated; nor could he gather any information as to their source from the peasants. They are said to grow in little clumps or granules on peculiar bushes found on the mountains just beneath the snow line.

Kern could not induce the kephir yeast to form spores. He explains the matter by saying that these yeast cells have for an infinity of generations grown in milk only, and have increased only by budding. Hence, when they are exposed to conditions favorable for spore formation, they are unable to form spores. He declares the kephir yeast to be ordinary *Saccharomyces cerevisiæ*, Meyen, saying that he cannot agree with Dr. Max Reess in classifying yeasts according to their form and size. The form and size of the cells vary too much; besides, the variations are not constant, being conditioned partly by age, partly by the nature of the nourishing medium, and partly by the temperature.

The other portion of the kephir-grain is made up of Bacteria embedded in a zoöglœa mass which is firm and elastic, comprising the bulk of the grain. The individual cells are short, cylindrical, and rod-shaped, $3.2\ \mu$ to $8\ \mu$ long and $0.8\ \mu$ broad, with homogeneous protoplasm. These cells increase by the regular splitting process characteristic of *Schizomycetes*.

The Bacteria in the zoöglœa are motionless; but in addition to these, when the kephir grain is placed in a nutrient solution, there are to be seen moving cells, exactly like the motionless ones in both form and size. By allowing these moving cells to dry upon a slide, then staining with *Extract Campech.* and removing the excess of the stain, Kern was able to demonstrate a very thin thread-like wavy cilium on but one of the ends of each cell.

Exposed to unfavorable conditions the Bacteria cells grow out into Leptothrix threads, varying from $10\ \mu$ to $40\ \mu$ in length, which are merely the necessary consequences of successive cell-division in which the products do not separate from each other. At various intervals in the length of such a thread agglomerations of protoplasm occur. At first there is hardly an indication of the splitting of such a mass, there being merely tiny incisions on either side; but these become larger and larger, until finally a single protoplasmic mass has given rise to two spores, separated by a regular cell wall. Thus it happens that in a Leptothrix thread each cell has two spores situated one at either end. Kern mentions still another kind of spore formation, seen in the individual cells, which differs markedly from that just described. In these cells spore formation begins with the appearance of a small bright point at each end of the cell. The points enlarge more and more, assume a well defined contour, and ultimately become true spores. The form is always round, the diameter never exceeding that of the mother cell before they are freed, but reaching $1\ \mu$ after liberation.

Kern names his kephir Bacterium *Dispora Caucasica*, nov. gen. et nov. sp., with the following distinguishing characteristics:—

(1.) The vegetative cells are in the form of short cylindrical rods, $3.2\ \mu$ to $8\ \mu$ long, and $0.8\ \mu$ broad.

(2.) In the zoöglœa condition the cells form white elastic clumps of considerable size.

(3.) The moving vegetative cells have on *one* end a thin, thread-like, wavy cilium.

(4.) The spores are round; when in the cells their diameter never exceeds that of the mother cell; when free, they may reach $1\ \mu$ in diameter.

(5.) The round spores are always two in number, one at each end of the cell.

From this subject Kern passes to his last topic, the power of resistance of the kephir-grains when subjected to external influences. Drying does not seem to deprive them of life. They contract a great deal, become dirty brown and hard as stone, so that they have even been called "little stones" or "pebbles" by the inhabitants of the Caucasus district. In this dried state they are kept for long periods of time, yet under suitable conditions they are always ready to cause fermentation again. Kern himself kept some for two months in his room. They were thoroughly desiccated, yet when placed in milk again they became gradually white, and in a few days could not be distinguished from fresh specimens. Under the microscope the dried clumps show that the yeast cells suffer most, very many being dead; the Bacteria seem to suffer very little, since they form spores.

Having thus summarized Kern's paper rather fully because it bears directly upon my subject, I am in position to describe an American milk-ferment which I hope to show is almost, if not quite, identical with the European kephir. The material which I studied consisted of two sets of specimens placed in my hands by Professor Farlow of Harvard University, to whom they had been sent by Dr. George Thurber, of Passaic, N. J., and Mr. J. Dearness, of London, Ontario. In both cases the specimens were in the form of rather small granules, very few being above a centimeter in diameter, of a dirty brown color, and presenting on their surfaces numerous lobes and fissures, thus reminding one of rather dirty gum-arabic. The material from Dr. Thurber was received in 1888, and at that time had already lain in a dried condition in his herbarium for several years. The specimens from Mr. Dearness, undistinguishable to the naked eye from those of Dr. Thurber, were received in January, 1891, under the name of "California bees' beer," with the note that "housekeepers through this country (Ontario) keep a self-sealing jar of this *Saccharomycete* half filled or more with sweetened water. The fermented product is drawn and drunk for a tonic."

The material from New Jersey and that from Ontario were practically identical in gross and microscopic characters, the Ontario grains being as a rule somewhat smaller, and the following description applies to both of them. In my experiments on the action in fermentation I used principally the New Jersey material, which, in spite of the long time it had been dried, revived when placed in a nutritive fluid.

I experimented with the Ontario material so far as to make sure that, like the New Jersey form, it caused a fermentation of saccharose; but in studying the fermentation of other sugars I used only the New Jersey form.

When soaked for a time in water, the grains become whitish, very firm and compact, and quite elastic. Examination under the microscope shows them to consist of two elements, a small proportion of yeast cells embedded in zoöglæa masses of rod-shaped Bacteria.

Although in the dried specimens the yeast cells seem entirely dead, yet when placed in a nutrient solution they begin to grow vigorously. They vary in size and shape, from elliptical to spherical, the average diameter of the latter being $4.2\ \mu$, and the former varying from $10.5\ \mu$ to $6.5\ \mu$ by $6\ \mu$ to $4\ \mu$. On careful examination, each yeast cell is found to have a plainly marked double contour, within which is an almost homogeneous protoplasm containing a small vacuole. Cultivation of the yeast cells in water increases the size of the vacuoles, and causes the formation of small fat globules at the poles; and cultivation in strong solutions of saccharose produces two or even three vacuoles in each cell, together with numerous fat globules. When such cells are mounted in a mixture of acetic acid and glycerine, the vacuoles disappear, and the protoplasm becomes finely granular.

The yeast cells increase by budding, growing best in solutions of dextrose and in milk, both of which they ferment; and it is in these substances that the best colonies are to be found. In pure water, the yeast cells for a short time increase slowly in numbers by budding, but no colonies are met with since the daughter cell separates from the mother cell as soon as it is formed. In cane-sugar or saccharose solutions, which the yeast is unable to ferment, the cells increase very rapidly in numbers, but it is hard to find a colony of more than three cells; whereas in milk and in solution of dextrose, colonies numbering at least from ten to fifteen cells are very common.

It was impossible to induce spore formation; and indeed the very fact that the yeast cells gave rise to new cells by the simple process of budding after they had been dried for several months seems to warrant the conclusion that there is no spore formation. That they are yeast cells, and not spores of *Mucor racemosus* or any other *Mucor*, is shown by the fact that not a particle of mycelium was found during the three months in which the yeast was under observation.

Kern decided his yeast to be a form of *Saccharomyces cerevisiæ*, Meyen. In the case of the North American kephir, the species evidently is not *S. cerevisiæ*, however much it resembles that species in

general appearance, for it cannot invert cane-sugar as ordinary beer yeast should do. Although I cultivated it in saccharose solutions of all strengths, it never caused a trace of fermentation. As soon, however, as I placed the yeast in a grape-sugar solution, i. e. a solution of dextrose, fermentation ensued. Unfortunately, Kern did not try the effect of his yeast upon saccharose, and we are therefore unable to compare his yeast with that found in American kephir in this important point. But the absence of information can hardly be urged as an evidence that the two forms are not the same. Again, ordinary beer yeast forms spores, while the kephir yeast does not, thus affording another reason for regarding them as distinct species.

Beyerinck has described the yeast which occurs in the Caucasian kephir grain in the "Centralblatt für Bakteriologie," Vol. VI. page 44, naming it *Saccharomyces kefyr* with the following distinguishing characteristics:—

(1.) The cells are of various sizes and shapes, from spherical to elliptical, the former measuring from $3.2\ \mu$ to $6.4\ \mu$ in diameter, and the latter varying from $3.2\ \mu$ — $9.6\ \mu$ in the major axis to $3.2\ \mu$ — $6.4\ \mu$ in the minor.

(2.) The yeast is associated with a rod-shaped Bacterium in a granular mass.

(3.) The yeast is not able to ferment saccharose or cane-sugar.

(4.) It is able to ferment lactose or milk-sugar.

(5.) It has no known spore formation. Since the North American yeast agrees with all these characteristics, while it differs in an important point from *S. cerevisiæ*, it will be sufficient for the present purpose if I apply the name *S. kefyr* to our American form without attempting to discuss at length disputed points in synonymy.

Let us turn now to the Bacteria. The cells are short cylindrical rods with homogeneous protoplasm, varying from $8.5\ \mu$ to $4.5\ \mu$ long by $0.8\ \mu$ broad; precisely agreeing with Kern's measurements. The cells increase by splitting perpendicularly to the long axis, the resulting cells being sometimes joined together, thus producing Leptothrix-like threads of all lengths, even to $120\ \mu$, and sometimes completely separated. Many of the isolated cells possess the power of motion, but after repeated efforts I was unable to demonstrate the presence of cilia.

It is not such an easy matter to induce these cells to form spores as Kern implies that it was in the case of his Bacteria. The best method is to place a clump of the yeast in a watch-crystal with a little water, covering the whole with another crystal. In twenty-four hours

Leptothrix threads, which seem to precede spore formation, begin to form, and within thirty-six to forty-eight hours the spores appear. It will be remembered that Kern gives two distinct methods of spore formation, — one occurring in isolated cells, and the other in the Leptothrix threads. It is no wonder, therefore, that neither method has received general credence. My investigations on the North American form have led to results diametrically opposed to those of Kern. First, I found but one method of spore formation; secondly, I found this method occurring only in the Leptothrix threads, although I sometimes found isolated cells bent or curled in such a manner that spore formation was well simulated. Spore formation in the Leptothrix threads takes place as follows. At each end of every cell of the thread a small bright dot appears, which becomes brighter, larger, and much more highly refractive than the rest of the cell, until finally it assumes a well defined spore wall and develops into a mature spore. Each cell has therefore two spores, one at each end, and each originating independently of the other. In no case did I see two spores formed, as Kern states, by the division of a single agglomerated mass of protoplasm into two portions.

There are two or three other important points in which the American alcoholic milk-ferment closely resembles the Caucasian kephir. So far as I know, no one has ever tried the experiment of making the North American yeast cause the alcoholic fermentation of milk. Struck with its gross and microscopic resemblances to kephir, I was induced to try the experiment, and to my pleasure I obtained alcoholic fermentation, the evolution of carbonic acid gas being sufficient to force the cork from the flask. I easily obtained a large precipitate of CaCO_3 from lime-water by the usual test for carbonic acid gas. The presence of alcohol was proved by the iodoform test. Since ethyl alcohol, $\text{C}_2\text{H}_5\text{OH}$, cannot be detected in the presence of lactic acid, $\text{C}_3\text{H}_6\text{O}_3$, (for lactic acid forms iodoform as easily as ethyl alcohol,) I neutralized with Na_2CO_3 after filtering off the fluid portion of the milk, distilling finally the neutralized filtrate. Thus every trace of lactic acid was removed. With KOH and iodine the distillate gave iodoform, thus proving the presence of alcohol. This fact alone, viz. that this yeast causes alcoholic fermentation of milk, is sufficient to establish a near relation to the Caucasian kephir. Moreover the fermented milk agrees closely with the description of the kephir drink. The milk does not sour in the ordinary sense, for it does not coagulate in large masses; still it is acid, contains some carbonic acid gas and alcohol, and is by no means unpleasant to the taste.

Another way in which this American milk-ferment resembles kephir is, that it causes alcoholic fermentation of dextrose. De Bary is authority for the statement, that the "kephir yeast, like its constituent the *Saccharomycete*, working by itself, gives rise to alcoholic fermentation in a nutrient solution of grape-sugar, though of a less active kind than that caused by beer-yeast."* The specimen which I had gave a good alcoholic fermentation with dextrose solutions, but caused no fermentation with saccharose. It seems, therefore, to have the power of fermenting only two of our natural sugars, — dextrose and milk-sugar.

When we consider the remarkable similarity of these American grains with the kephir granules in color, shape, and general appearance; the great similarity between the yeast cells and bacteria of each in appearance, habits, mode of growth, form, and size; the fact that both of these yeasts cause alcoholic fermentation of milk; the fact that the drink formed by the American kephir closely resembles the description of kephir; the minor resemblance between the two, that of fermenting dextrose solutions, and that of its great capacity for resisting external influences, — we are justified in concluding the American milk-ferment to be a very near relative of the European kephir, if it be not indeed identical with it.

One point remains, viz. How can this yeast cause alcoholic fermentation of milk-sugar? This question, which did not present itself to Kern, De Bary has tried to explain in his 'Lectures on Bacteria.' Speaking of kephir, he says, "The changes in the milk which produce the drink here described are brought about by the combined activity of at least three ferment-organisms." There is the yeast cell, the *Bacillus* of the kephir-grain, and the *Bacterium* of lactic fermentation. He goes on to say that "the acidification is caused by the conversion of a portion of the milk-sugar into lactic acid by the bacterium of that acid. The alcoholic fermentation, that is, the formation of alcohol and of a large part at least of the carbonic acid, is indebted for its material to another portion of the milk-sugar, and for its existence to the fermenting power of the *Sprouting Fungus* (yeast). . . . But alcoholic fermentation is produced in milk-sugar as such neither by *Saccharomycetes*, with which we are acquainted, nor, as experiment has shown, by those of which we are speaking. To make this fermentation possible the sugar must first be inverted, split into fermentable kinds of sugar." De Bary continues: "According to Nägeli, the for-

* Lectures on Bacteria, De Bary, translated by Garnsey and Balfour, p. 96.

mation of an enzyme which inverts milk-sugar is a general phenomenon in Bacteria, and Hueppe has shown that it is probable in the case of his *Bacillus* of lactic acid in particular." De Bary then concludes: "The inversion required in this case to enable the *Saccharomycete* to set up alcoholic fermentation is the work therefore of the *Bacillus* of lactic acid, or of the *Bacterium* of the *Zoöglæa*, or of both."

But De Bary has since revoked this explanation. A. Levy of Hagenau discovered that kephir may be made without any kephir grains "simply by shaking the milk with sufficient violence while it is turning sour. A trial convinced me," says De Bary, "of the correctness of this statement. The kephir obtained by shaking was not perceptibly different in taste or other qualities from the kephir of the grains, and the determination of alcohol, kindly made for me by Professor Schmiedeberg gave 1 per cent in some specimens of the former kind and 0.4 per cent in one of the latter; sour milk not shaken contained no trace of alcohol or only a doubtful one. Our former explanation, therefore, must be abandoned, and there is no other ready at present to take its place."

Beyerinck has also proposed a theory to account for alcoholic fermentation of milk. There are at present, he says, four yeasts which are known to cause such fermentation: (1) that of Duclaux,* (2) that of Adametz, called *Saccharomyces lactis*,† (3) that of the kephir called by Beyerinck *Saccharomyces kefyr*, and (4) *Saccharomyces Tyrocola*.‡ As a matter of fact there is one other which he overlooked, *Saccharomyces galacticola*, described by Pirota,§ of which I intend to speak later. Beyerinck supposes that these yeasts secrete an enzyme which he names *lactase*, since it inverts lactose or milk-sugar, and which he declares to be in every way analogous with invertine. The inverted milk-sugar is next acted upon by the yeast, carbonic acid gas eliminated, and alcohol formed. Hence, if his supposition be true, *Saccharomyces kefyr* should ferment sweet milk by first producing its enzyme and then by acting upon the inverted product; but it is universally agreed by all who have written upon kephir that the lactic acid fermentation *must* precede the alcoholic, or else the latter will not take place. Beyerinck's theory therefore fails, in that it pays no heed to the Bacteria of lactic fermentation.

* Ann. d. l'Inst. Pasteur, 1887, I. 573. See Ibid., 1889, III. 201.

† Centralblatt. f. Bakt. u. Parasit., V. 116.

‡ Ibid., VI. 44.

§ Pirota et Rib. Studi sul Latte. Pavia, 1879.

I have experimented with our North American ferment and find the facts to be these: (1.) It causes alcoholic fermentation of milk-sugar or lactose, $C_{12}H_{22}O_{11}$. (2.) It causes fermentation of dextrose, $C_6H_{12}O_6$. (3.) It does not cause fermentation of saccharose or cane-sugar, which has the same empirical formula as lactose. In addition to these three facts it is also known, as Hueppe has shown, (1) that the *Bacillus* of lactic fermentation causes to some extent the inversion of milk-sugar; (2) that lactic acid, according to Hammarsten, by standing with milk-sugar inverts it to dextrose and galactose just as does any mineral acid; (3) that the *Bacillus* of lactic fermentation acts further on the galactose, $C_6H_{12}O_6$, converting it into two molecules of lactic acid, $C_3H_6O_3$.

From these data it seems evident that alcoholic fermentation of milk takes place in the following manner. The *Bacillus acidilactis* begins the process by forming some lactic acid, which in turn, assisted by the *Bacillus* itself, inverts the milk-sugar to galactose and dextrose. The galactose is further acted upon by *Bacillus acidilactis* and converted into lactic acid; the dextrose is acted upon by the yeast, and converted into alcohol and carbonic acid gas. In the kephir drink, therefore, we should find plenty of lactic acid, a little milk-sugar, not inverted, the amount depending upon the duration of fermentation, some alcohol, and carbonic acid gas, — precisely what is found.

One vital objection may, however, still be urged against this theory. If it be true, as I have said, that the *Bacillus acidilactis* to some extent, and the lactic acid to a greater extent, cause the inversion of milk-sugar, then should not ordinary beer yeast, *Saccharomyces cerevisiæ*, Meyen, cause alcoholic fermentation in sour milk, since the milk-sugar, according to the theory, must be here inverted to fermentable dextrose and to galactose? It should cause such fermentation; and if it does, the theory is confirmed. Upon experiment, I found that ordinary beer yeast when added to sour milk or to milk on the point of souring did cause fermentation, much carbonic acid gas being eliminated and some alcohol formed; sweet milk, however, did not ferment with beer yeast.

It will be observed that I have given no function to the Bacteria of the kephir granules. The very fact that they remain almost wholly in the *Zoöglœa* masses during fermentation, comparatively few going out into the milk, seems to indicate that they have little to do with this alcoholic fermentation; and this is made still more probable by the additional fact that, though absent in the fermentation of sour milk by beer yeast, still fermentation ensues.

To this theory De Bary has objected that kephir can be made simply by shaking milk which is on the point of souring, such kephir being called Pseudo- or Schüttelkephir. He refers to a paper by A. Levy,* of Hagenau, in which Levy claimed that ordinary sour milk shaken in a flask with eight or ten parts of cold boiled milk at about 10° R. gave carbonic acid gas, lactic acid, alcohol, and peptone. But Levy says, by shaking, the air is introduced, and the fermentation and peptonization are probably brought about by micro-organisms, which are very numerous in milk. Franz Kogelmann † has also published a method for obtaining kephir easily and cheaply. Take one volume of ordinary buttermilk, shake with two of fresh, and there is obtained a fluid "*identical* with kephir," containing carbonic dioxide, alcohol, lactic acid, casein, etc. Notwithstanding these claims, there is some doubt about the identity of Schüttelkephir and Kogelmann's kephir with the true sort, as Rudeck's ‡ table shows.

	Milk. One Litre.	Kogelmann's Kephir.	Pseudo Kephir.	True Kephir, 36 hours.
Casein	48.00	35.00	38.00	36.50
Butter	38.00	11.00	16.00	18.00
Lactose	41.00	9.00	13.00	18.00
Lactic acid	14.50	11.00	6.00
Alcohol	Trace	5.00
Albumen	1.80	"	1.50
Peptonized albumen	0.90	"	2.00
Lactosyntonid	0.40	0.80
Peptone	Trace	0.48
Salts and Water . . .	871.20	929.20	922.00	911.72
Totals	1000.00	1000.00	1000.00	1000.00

Despite this table, however, it is not improbable that alcoholic fermentation often does actually take place in Kogelmann's and Levy's methods, as the following paragraph may show.

* Die wahre Natur des Kefirs. Deuts. Med. Ztg., 1886, p. 783.

† Ueber Milchwein (Kefir). Ibid. See also Pharm. Cent. Halle, XXVII. 42.

‡ Pharm. Ztg. Berl., XXXIII. 426.

Levy and Kogelmann were by no means the first to experiment on this subject. Blondlot,* as early as 1872, found that he could obtain alcoholic fermentation in milk simply by shaking it. Pirotta investigated this fermented fluid, and found a yeast, which he named *Saccharomyces galacticola*, identical with *Saccharomyces cerevisiæ* in appearance, size, spore formation, and in the fact that both ferment sour milk. It is not improbable, therefore, that this yeast may be nothing more nor less than *Saccharomyces cerevisiæ* itself, and that ordinary beer yeast is one of the micro-organisms which sprung up in Levy's kephir, of which he unfortunately omitted to make a microscopical examination. Hence De Bary's objection that sour milk, simply shaken, will give alcoholic fermentation, loses its significance.

Throughout Germany and Russia kephir has become a very celebrated drink, simply because a considerable portion of the albuminoids of the milk are peptonized. For persons of weak digestion, for children, and for dyspeptics generally, it is an excellent diet, since it relieves the stomach of much of its work. Hence the fame of kephir has spread far and wide, and a kephir factory has been started at Hamburg. The following table, taken from J. Biel's "Ueber die Eiweisstoffe des Kefirs," shows this peptonization very neatly.

In 100 parts of kephir were obtained : —

	Kephir fermented One Day.	Kephir fermented Two Days.	Kephir fermented Three Days.
Lactic acid	0.540	0.5625	0.6525
Lactose	3.750	3.2200	3.0940
Casein	3.340	2.8725	2.9975
Albumen	0.115	0.0300	0.0000
Acid albumen	0.095	0.1075	0.2500
Peptonized albumen . . .	0.190	0.2815	0.4085
Peptone	0.035	0.0460	0.0815

From the table it is evident that the casein and albumen decrease during fermentation, while the peptone, peptonized albumen, and acid albumen increase. This is shown still better by another table.

* Comptes Rendus, LXXIV. 534.

In 100 parts of albuminoids were obtained : —

	Kephir fermented One Day.	Kephir fermented Two Days.	Kephir fermented Three Days.
Casein	88.47	86.07	80.20
Albumen	3.05	0.90	0.00
Acid albumen	2.52	3.22	6.69
Peptonized albumen . . .	5.03	8.43	10.93
Peptone	0.93	1.38	2.18

An analysis of the milk fermented by the American yeast shows the presence of peptone, in some quantity, whereas sour milk fermented by beer yeast gave only a trace, thus agreeing with Rudeck's analysis for Kogelmann's kephir. From these analyses there is but one inference, — the peptonizing power must lie, not in the *Bacillus acidilactis*, which is common to all these true and false kephirs, but in the yeast which Beyerinck has named *Saccharomyces kefyr*, and which exists in the United States.

In conclusion, I would return my thanks to Prof. W. G. Farlow and Prof. H. B. Hill, for advice given during the progress of my work.

